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Review / *Derleme*

What has been done in the fight against *Varroa destructor*: from the past to the present

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Abstract: Bees are the major pollinators in natural ecosystems and in the agricultural production of several crops used for human consumption. However, they are exposed to multiple stressors that are causing a serious decline in their population. We highlight a major one among them, the *Varroa destructor* mite (Varroa) that causes severe impacts on the health of honey bee colonies, transmitting a variety of viruses that can affect the survival ability of individual bees and entire colonies. Diagnosis and mite control methods have been intensively studied in recent decades, with many studies in different areas of knowledge having been conducted. This overview summarizes these studies with a focus on colony defense systems, biological characteristics of the parasite Varroa, diagnostic methods used to establish the infestation level of colonies, and currently used control methods.
Keywords: Diagnosis, Honey bee disease, mite, parasite, *Varroa* control.

Geçmişten günümüze *Varroa destructor* mücadelesi

Özet: Arılar, doğal ekosistemlerde ve insan tüketimi için kullanılan çeşitli mahsullerin tarımsal üretiminde başlıca tozlayıcılardır. Bununla birlikte, popülasyonlarında ciddi bir düşüşe neden olan çoklu stres faktörlerine maruz kalırlar. Bal arısı kolonilerinin sağlığı üzerinde ciddi etkilere neden olan ve tek tek arıların ve tüm kolonilerin hayatta kalma kabiliyetini etkileyebilecek çeşitli virüsler bulaştıran *Varroa destructor* akarı (Varroa) üzerinde duruyoruz. Teşhis ve akar kontrol yöntemleri, son yıllarda yoğun bir şekilde araştırılmış ve farklı bilgi alanlarında birçok çalışma yapılmıştır. Bu derleme, koloni savunma sistemlerine, parazit Varroa'nın biyolojik özelliklerine, kolonilerin istila düzeyini belirlemek için kullanılan tanı yöntemlerine ve şu anda kullanılan kontrol yöntemlerine odaklanarak bu çalışmaları özetlemektedir.
Anahtar sözcükler: Akar, Bal arısı hastalığı, Parazit, Teşhis, Varroa kontrolü.

Introduction

Bees are exposed to numerous stressors and the decline of their populations on different continents has been reported in recent decades (13, 84, 87). According to Goulson et al. (42), there is evidence of a combination of factors causing this decline, such as: pesticides, habitat degradation and fragmentation, increase of monocultures in agriculture, decline in flower diversity and abundance, as well as parasites and diseases. Among the stressors, *Varroa destructor* mite the main pest that affects the health of young and adult bees and, consequently, the colony (11, 28, 47, 95).

In accordance with Milani (78), in the second half of the last century, the mite *V. destructor* spread across the European continent and since 1980 several studies have been conducted in order to find methods to identify and control the mites (16, 69). This review aims to compile knowledge about the *V. destructor* mite from recent literature, such as its biological characteristics, impact on bee populations, and updated control mechanisms.

Honey bee colony defense systems

Honey bees have evolved a collective anti-parasite defense system which is allo-grooming behavior in order to remove parasites from group members (101). Social defense systems in honey bees can be activated on demand, based on collective action or altruistic behaviors of infected individuals that benefit the colony (23). Individual bees evolved physiological, immunological, and behavioral defense systems toward pathogens and parasites. The colony-level response, as opposed to the immune responses within an individual bee, presents coordinated behavioral cooperation among individual bees in a nest. The collective defense against parasites is termed "social immunity" (24). We can observe continuous simple interaction between adult honey bees in a colony. Social immune responses against parasites are initiated on a colony level when several individual adult honey bees interact with each other. Social immunity with a defense effect is a type of behavioral mechanism believed to contribute to reduced Varroa survivability in honey bee colonies. The system targets the mites at the phoretic stage, during their feeding on adult host bees. It occurs when adult worker bees remove mites from adult bee bodies via auto-grooming and allo-grooming. An important target of social immunity is during the mite reproduction phase on bee pupae sealed in comb cells (49).

Tolerance and resistance characteristics

When we select and rear honey bees two professional terms need to be considered and potentially introduced into breeding practice. They are tolerance and resistance, which refer to different mechanisms to enable bees in reducing the effects of the parasite Varroa or any pathogenic or other physiological factors, especially with repeated exposure. The body thus becomes less responsive and develops the ability to overcome the effects without exhibiting disease appearance (27). In a honey bee apiary, tolerance is the ability of bees to live in association with Varroa. When Varroa transfers secondary infections, bees may be developing resistance to these pathogenic organisms and the final effect is developing tolerance to Varroa infestation (14, 59). The ability of organisms to remain unaffected or slightly affected is thus considered as resistance. Resistant bees are able to maintain low levels of Varroa infestation or other pathogens due to known or unknown bee characteristics. In absolute resistant bees, Varroa or other pathogens would not infest or infect the individual bee or the entire colony.

Grooming behavior of honey bees

The ability to remove foreign particles from the honey bee body surface can be removed by performing self-grooming behavior (auto-grooming). The phenomenon when bees groom another bee it is known as allo-grooming. This behavior was described in *Apis cerana* as a defense mechanism against Varroa (15), and by Africanized *A. mellifera* in the tropics (79). One of the possible mechanisms of resistance to Varroa in Africanized bees is "auto- and allo-grooming" behavior, where bees brush particles from themselves or from their nest mates. It is demonstrated that Africanized bees, which are important hybrids of *A. mellifera scutellata* nowadays bred in Brazil, appear to have more resistance than European strains. Another example of grooming behavior efficacy as a defense mechanism to Varroa is described in *A. cerana* (79). Adult workers grooming behaviors can explain reduced *V. destructor* reproductive success in the colony and this phenomenon has an impact on colony survival. Varroa resistant colonies with expressed adult bees grooming behavior have lower mite population rates (up to 15-fold) and a higher percentage of damaged mites (up to 9-fold) in comparison to colonies with weak grooming behavior. It has been established that grooming behavior and corresponding Varroa mite populations growth is an important component in the resistance of some honey bee genotypes (49).

Hygienic behavior of honey bees

Disease resistance is known to correlate with the "hygienic behavior" of worker bees. This is a genetically controlled collective response by adult workers to recognize and then remove the dead, infected or damaged brood (larvae and pupae) (43). Hygienic behavior was originally described in honey bee colonies resistant against *Paenibacillus larvae*, where bees may uncap comb cells containing dead, sick or damaged brood and remove this brood from the colony (99). It was found that hygienic behavior is also a significant defense mechanism against Varroa mites parasitism (10, 61, 108, 124). The hygienic behavior of honey bees is therefore responsible for the ability to identify and remove Varroa infested pupae from comb cells in the honey bee colony nest. *A. cerana* worker bees in their colonies regularly detect Varroa-infested pupae. Workers may either make a hole in the wax capping or may remove the pupa and subsequently release the mite confined in the brood cell (10). This resistance mechanism is well known in *A. cerana* and may also be activated in heavily parasitized host *A. mellifera* colonies. Varroa reproduction preferably takes place in drone broods and it is thought that *A. cerana* colonies resistance largely depends on the seasonal nature of their drone development (35, 97).

Rapid population growth of Varroa in colonies of European honey bee races is due to Varroa’s ability to reproduce in both drone and worker brood of *A. mellifera*. Hygienic behavior based on the workers olfactory stimuli is responsible for their ability to remove mite-infested worker brood. This trait is a highly desirable selection criterion against varroosis (77, 107). By killing any mite offspring during the reproductive cycle and reducing Varroa reproductive success there is a negative cumulative effect on the Varroa population dynamic in colonies. In selected colonies, with highly Varroa infested pupae, workers were able to remove up to 60% of the experimentally infested brood (106). Adult worker bees detecting and removing mite-infested brood have been defined as displaying Varroa sensitive hygiene (VSH) and this has been identified as a trait in specific bee strains (53).

These colonies were selected to effectively remove pupae that were mite-infested (52). The VSH selection procedure based on brood removal is more effective in comparison to the freeze-killed brood assay procedure (62). The removal of infested brood inhibits individual mite reproduction and therefore reduces the in-colony entire mite population (54-55). VSH selected bees expressed the specific characteristic that Varroa is removed from comb cell opening after worker's bee hygienic activities (53). There is a need to study the variety of potential resistance mechanisms in honey bee colonies that can contribute to colonies having a lower level of Varroa infestation. In beekeeping practice, specific phenotypic characteristics have been observed and through selection activities more Varroa tolerant honey bee lines can be reproduced.

Impact of Varroa on colonies health and survival of honey bees

Varroa as an external parasite has physical and pathological effects on individual bee and on the whole colony level. Varroa attacks both adult bees and developing larvae. Parasitized brood is injured with reduced larval protein content and subsequently bee body weight is reduced, organ development is affected and finally, worker or drone life is shortened (11). Emerging bees may be deformed with missing legs or wings and together with deformed wing virus, microbes, and reduced immune competency, adult bees' survival is significantly affected in untreated colonies. Highly infested colonies that are not examined for mites, and effectively treated may die or contribute to increased winter mortality or queenlessness. Varroa mites are also a vector in transmitting a number of viruses from infected to healthy bees. Viruses associated with Varroa mites in colonies are: DWV, Acute Bee Paralysis Virus (ABPV), Chronic Bee Paralysis Virus (CBPV), Slow Bee Paralysis Virus (SBPV) (76), Black Queen Cell Virus (BQCV), Kashmir Bee Virus (KBV), Sacbrood Virus (SBV) (75, 76, 111).

Varroa parasitization, together with viruses as secondary infections, influences the weakened bee's immune system and contributes to an increased risk of colony mortalities. *Varroa* infestation has been shown to induce increased DWV abundance in parasitized bees and also result in increased immunosuppression (82). Consequentially ABPV, BQCV and DWV also appear in workers and queens during queen rearing procedures (127). The increased incidence of viruses present in bees has resulted also in the appearance of an epidemic that is demonstrated by increased colony mortalities and this has been contributed to by *V. destructor* parasitizing (123). Bees are thus directly damaged by mites feeding on them, and additionally efficiently inoculated with harmful viruses. It was also shown that highly pathogenic DWV and ABPV associated with Varroa in highly infested honey bee colonies contribute to winter colony losses (8). Moreover, it has been found that the mites are activators of several mechanisms in honey bees to induce immune-suppressive action and subsequently increase virus replication (126). Therefore, proper Varroa diagnosis and timing of colony treatment together with sufficient efficacy is imperative for beekeepers to preserve their honey bee stock.

Reproduction and infertility of Varroa

Varroa mites reproduce more in done brood than in worker brood, which may be because of physiological factors of non-reproduction mites observed only in worker cells. A female Varroa has on average 1.5 - 2 reproductive cycles in its life (37) with a range of 0 - 7 cycles (100). It was found that Varroa oviposition after entering into brood cell might be stimulated by prior feeding on adult bees or the bee larvae respectively and a shorter feeding period has slightly reduced fertility potency in female mites.

The phoretic mites are more attracted to nurse than forager bees probably because they carry them to their reproduction site (25). Duration of the phoretic phase is variable and could depend on the type of bee carrying the mite having an impact on the mites' life cycle and reproduction. The phoretic mites stay on adult bees for a variable amount of time, from one to ten days (7). Multiply-infested cells also have an impact on reduced Varroa reproduction as a result of the existence of chemical factors in female Varroa and subsequently, the number of daughters per mite decreases in multiply-infested cells (83). It is also evident that Varroa has lower reproduction potential in tropical Brazil where bees expressed approximately two times greater proportion of non-reproductive mites in comparison to honey bees in a temperate climate in Europe (94). In addition, mite population dynamics depends also on the type of brood (i.e., worker drone). The honey bee drone brood is more attractive to *V. destructor*, in comparison to worker brood. Drone brood takes more time to develop and therefore leads to the higher production of Varroa offspring (39). Mother mites are able to choose nurse bees over foragers and newly emerged bees as their optimal host in the phoretic phase to quickly infest new brood cells (125). Varroa mite population can increase in honey bee colonies up to ten times during the beekeeping season and thus demonstrate a high degree of adaptation (105).

Over a long period of time, with the help of selected breeding activities, the host has the opportunity to develop resistance mechanisms to its pest. There is some evidence in beekeeping operations indicating variability between honey bee colonies in resistance of honey bees to Varroa. In addition to resistance traits of individual larvae, VSH behavior of workers has been demonstrated as a useful indicator for developing honey bee resistance to Varroa mites in breeding stocks. This behavior can be considered in breeding activities where selected honey bees show the ability to detect infested capped brood and destroy Varroa. Strains of VSH bees have been developed and are now successfully used in beekeeping operations in the United States of America and are available for individual beekeepers (51, 121).

It was also found that *A. mellifera carnica*, *A. m. mellifera*, and *A. m. ligustica* have developed no adaptations in terms of the reproductive success of Varroa. In all subspecies groups mother mites reproduce equally successfully and are potentially able to cause detrimental damage to their host when not treated sufficiently. Furthermore, it was also established that a population once Varroa tolerant does not necessarily pass on this trait to following generations. Established tolerance to Varroa parasitism is also not evident in offspring (F1) (86). This phenomenon could be of particular interest for beekeepers when selecting populations for resistance breeding.

Breeding better bees

Numerous parameters have been considered and applied in apiculture practice in order to contribute to increased Varroa resistant bees such as: the initial population of mites, duration of brood developmental and the capped period of workers and drones. Additionally Varroa phoretic period, Varroa preference to drone brood, Varroa infertility level, the number of Varroa reproductive cycles, and winter mortality of Varroa are also considered for selection (35). Beekeepers can also incorporate into their breeding program additional variables associated with Varroa parasitism, such as colonies survival during winter period, monitoring natural Varroa mortality and Varroa population growth in the host colony. In North America breeding programs have been performed in order to produce Varroa resistance stocks using imported honey bee races (60). In European beekeeping conditions, the rich diversity of natural honey bee races (subspecies) and local varieties (ecotypes) offers enormous genetic resources for selection with native honey bees on Varroa resistance. The most important autochthonous subspecies employed for selection programs are *A. m. carnica*, *A. m. ligustica*, and *A. m. macedonica,* among others widely spread throughout Europe. Furthermore, in addition to selection on traits affecting the reproductive rate of Varroa, which is considered as the most important trait, selection on grooming and hygienic behavior would contribute to a reduction in Varroa population. Varroa resistant stock need to be continuously maintained with a sufficient gene pool and controlled mating is required for achieving progress in developing better honey bee colonies. Selected honey bee colonies normally require fewer acaricide treatments for Varroa control, and they demonstrate longer survival without Varroa control. Some honey bee populations in Europe have been found to naturally survive mite infestations without treatment for several years. Surviving colonies originating from Varroa infested colonies were able to survive several years without treatment (36, 71, 85). The mechanisms of naturally developed resistance are not described yet. Potentially reduced mite reproduction in naturally surviving colonies may be monitored in normal beekeeping conditions (71, 92).

Varroa control

The world's honey bees are in a huge decline, with millions of hives disappearing in recent years (44). This decline is of growing concern on account of the critical role honey bees play in maintaining natural plant communities and sustaining human and livestock food sources. Today, it is linked to multiple environmental stressors including but not limited to virus, bacteria or a parasitic infection, pesticide exposure, and poor nutrition, environmental variation, and the synergistic effects between these factors (42, 103, 112). It is known that Varroa can be devastating for honey bee colonies and no other honey bee pathogen or parasite has had a comparable impact on managed honey bees. Therefore, Varroa control has been an important part of maintaining the colony's health. Determination of Varroa infestation in honey bee colonies with an appropriate method that beekeepers can use to measure Varroa infestations in their hives is important for efficient mite control. There are various methods including genetic, mechanical, biological, and chemical for Varroa control. However, there is no single best method to control the Varroa population (Figure 1) (5, 98).

Mechanical control

Mechanical controls (Cultural or physical treatments) involve hive manipulations and interruption of the brood cycle in the management practices of beekeeping. These methods could be used as an alternative method based on not using chemicals to reduce mite levels. Due to their low effectiveness against Varroa, they must be used in combination with other control techniques each working in different ways (chemical treatment) in the

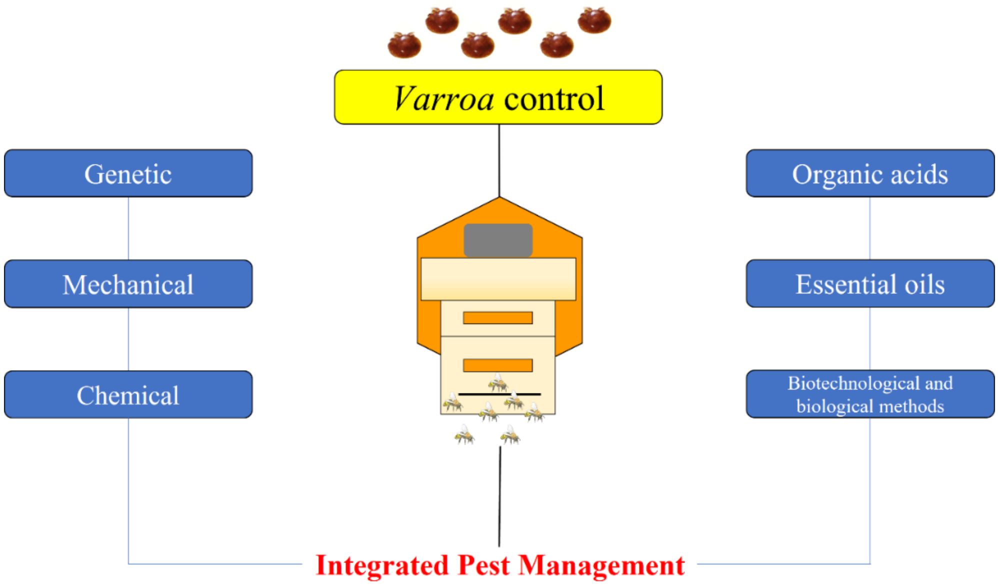

Figure 1. The design of different methods of Varroa control.

management practices of beekeeping. Mechanical controls include drone brood removal, screened bottom boards, dusting bees with powdered sugar, and manipulating brood (47). The most effective cultural and physical method is the partial removal of drone brood, where mites reproduce more often in comparison to worker broods, with average differences between five and 12-fold (21, 22). However, this method may not be practical for beekeepers who manage a large number of beehives as it is labor intensive. Also, this method can be only used in spring and summer when drone brood is present in the beehive (117). Drone brood removal significantly reduces the population of mites in colonies. However, it may not be as effective as chemical-based methods. Thus, it can serve as a useful component in an integrated Varroa control program and may reduce the need for other treatments on a colony-by-colony basis (17).

Other mechanical methods include screened bottom boards, and powdered sugar dusting. Sprinkling several non-toxic powdered materials, including glucose powder, ground pollen, wheat flour and baby powder, on honey bees stimulates grooming behavior which enables them to bite adult mites, and remove the mites from their bodies, resulting in the reduction of Varroa population in bee hives. However, it requires large quantities of high-priced materials and intense manipulation in beehives and leads to increased bee mortality. Also, it is not sufficient Varroa mite control on its own but can be used in conjunction with screened bottom boards for higher effectiveness (30, 32). Screen bottom board (sticky bottom board or open mesh floor) tray inserted from the back of the bee hive is used to reduce mite populations by preventing the return of Varroa, that fall from the bee cluster as a result of grooming or any other reason (73). This may be due to providing improved hive ventilation or the loss of mites falling to the bottom of the hive through the screened floor (98). Replacing solid wooden floor (Solid surface) with screened bottom boards (non-solid surface) in bee hives makes Varroa less likely to climb back or invade brood cells, resulting in a reduction in Varroa reproduction in brood cells (70). As a result, these mechanical methods show little effectiveness and should only be used as a supplement to more effective methods like chemical treatments.

Chemical control

Today, chemical control is of great importance to reduce the Varroa mite population in honey bee colonies and can be achieved by the use of various acaricides. All acaricidal chemicals used for controlling Varroa mites are called "Varroacides". Synthetic acaricides including pyrethroids (tau-fluvalinate, flumethrine), formamidines (Amitraz), and organophosphates (Coumaphos) have been the major effective method used for years in the control of Varroa (1, 5). As a result of the widespread use of chemical-based drugs for Varroa control the beekeeping industry is facing two important public health issues. Firstly, the Varroa mites develop resistance to these chemicals when used repeatedly. The second major problem is the presence of chemical residues in bee hive substrates and products (97, 104). An increase in these problems has raised interest in treatment with non-synthetic substances. These control strategies are mostly based on the use of organic acids (formic acid, oxalic acid, lactic acid) and volatile oils (thymol, carvacrol and menthol) (88, 115). Due to their hydrophilic and volatile properties, they are unlikely to accumulate in the stored honey, where they are able to migrate from the wax comb. Also, mites are unlikely to develop resistance to them (50, 97). However, single applications of organic acids may be insufficient for adequate Varroa control. Whereas chemical control, combined with mechanical control, can increase the efficacy of Varroa control (47).

Synthetic acaricides: In the last 20 years, the most commonly used synthetic acaricides against *V. destructor* are the organophosphate coumaphos, the pyrethroids tau-fluvalinate and flumethrin, and the formamidine amitraz (72). Coumaphos has been widely used for many years as an active ingredient to control ectoparasites on cattle, goats, sheep, and honey bees. Veterinary medicinal products (Checkmite, Perizin) containing coumaphos impregnated in plastic strips have used to control Varroa in the beehives by hanging them between frames for the allotted time, which is approximately 45 days. It is a phosphonothioate proinsecticide requiring in vivo bioactivation by cytochrome P450 monooxygenases to its active phosphate metabolite coroxon, which is selectively toxic to insects through inhibition of acetylcholinesterase (AChE) but far less toxic to mammals (65, 120). It had high varrocidal activity against Varroa in the first years of its use, but recently drastic decreases in varrocidal activity have occurred. The reduction in its efficacy has been proposed to be related to the development of resistant strains of Varroa (56).

The synthetic pyrethroid acaricides flumethrin (Bayvarol) and tau-fluvalinate (Apistan) are registered treatments against Varroa in most countries in the world (2, 104). They affect both the peripheral and central nervous system of the insects by modifying the ion channels (especially sodium channels) in neuronal membranes. They also induce excessive sensory hyperactivity of the peripheral nervous system and muscle spasms (57, 110). They have relatively low toxicities to honey bees and are easy to use. Plastic strips impregnated with tau-fluvalinate and flumethrin are inserted between combs in bee hives. While synthetic pyrethroids initially showed high acaricide performance, individuals resistant to these acaricides appeared due to the adaptation potential of the mite (41, 56, 67, 109). Amitraz, a formamidine acaricide proposed to activate octopamine/tyramine receptors, is widely used to control mite and tick infestation of domestic animals. Amitraz impregnated strips have been used for Varroa control in honey bee colonies for over twenty years (48, 68). Extensive use of amitraz has resulted in high levels of resistance in some areas and their treatment failures (93).

Organic acids: Overuse and misuse of synthetic acaricides have caused the development of resistance in Varroa populations, therefore, beekeepers have turned to alternative treatments (104). As alternatives to conventional treatment methods using acaricides, organic acids such as formic acid and oxalic acid, and essential oils such as thymol are available for Varroa treatment (46, 115). Organic acids such as acetic, citric, formic, oxalic and many others are present in honey’s composition in small quantities and can play an important role in controlling Varroa infestation (102). Oxalic acid and formic acid are the most widely used organic acids for Varroa control with lactic acid use less common. There are significant differences between these acids in terms of application, concentration and amount used. Although many commercial preparations are available, beekeepers apply them empirically using different methods whose efficacy has not been tested. Therefore, care should be taken when applying untested and non-commercial methods (116, 118).

Oxalic acid, which is an organic compound with the formula C2H2O4, in the form of crystals, gelatin capsules or tablets is heat-evaporated and used predominantly during the broodless period (91). Using a syringe or similar applicator, oxalic acid dihydrate is normally trickled directly on the bees in the spaces between the combs. The application is quick, cheap, and easy (113). Different concentrations of oxalic acid have been used for control of Varroa mites due to the climatic conditions of the European regions. Notably, a higher concentration of oxalic acid was shown to be more effective in a southern climate (80, 81). In a northern climate, a lower concentration of oxalic acid was found to be more suitable (38, 91).

Formic acid, which a colorless liquid that fumes, is effective both against phoretic and reproductive phases of the mites (90). It was approved for use on mites in gel-pack formulation (pad) and liquid formulations being administered in bee hives using different evaporators. The pad containing formic acid is applied by placing it on the upper bars of the brood box. As a fumigant, formic acid vapors generated by heating to a high temperature are released into the beehive (4, 33). The effectiveness of formic acid for the control of Varroa varies considerably based on several factors such as the distance from the place where formic acid is applied to the honeycombs, the place where it is placed in the hive, the amount of brood in the hive, time of year, and the ambient temperature (18, 29, 116).

Essential oils: Essential oils, extracted from various parts of medicinal and aromatic plants using several methods such as hydrodistillation or steam distillation, are complex mixture of volatile aromatic compounds (6). Essential oils represent an alternative and useful tool to control Varroa due to having high toxicity to mites, low toxicity to bees and mammals, and a low environmental impact. Essential oils can be incorporated into an Integrated Pest Management program, reducing the use of synthetic drugs (62). While many essential oil components, including thymol, carvacrol, citral, menthol, and Tau-ßuvalinate, have varroacidal activity, thymol is an ingredient that has rapidly risen in popularity (115). Active ingredient Thymol (Thymovar, Apiguard), is a product of thyme oil. It is an essential-oil based product effective against Varroa and exerts its bioactivity in a way that is most likely mediated by neurological mechanisms (9). It can be used both alone or in combination with other varroacidal agents and biotechnological applications such as brood removal (19, 45). Thymol is used in the form of tray (coated slow-release gel) and impregnated cloth sponge strip for the control of Varroa.

Biotechnological and biological methods

There are several biological ways for control and treatment of *V. destructor*. These are investigated for biocontrol of honeybees against some ectoparasites including *V. destructor*.

Biotechnological approach: This method is a combination of mechanical technique and application of organic molecules. The main aim is to reduce the population burden of *V. destructor* with using total interruption of brood, removing all drone brood, caging the queen and adding trapping combs (16, 40, 91). Unfortunately, these methods are not effective in every region in the world. For instance, interruption of brood has different results for Europe and USA (63). The methods should be reconsidered according to the regions. Sugar shake, which is another method used for diagnosis, decreases the pressure of the number of mites on the colony without any serious harm. Although these are effective methods to reduce the population pressure of *V. destructor*, they are not desirable because they can lead to honey losses and are difficult to apply.

Predator Animals: Predators in nature can be another alternative in the treatment of *V. destructor* biologically. For this, various species have been investigated for their potential benefit in treatments. One of these beneficial species is pseudoscorpions which feed on ectoparasites in hives causing no harm to honeybees (31, 119). They use their venom to paralyse and kill the mites (122). Different kinds of species have been identified, such as *Nesochernes gracilis* and *Chelifer cancroides*. Although some of them are beneficial to bees, this does not mean that all can have beneficial effects on the colony. Pseudoscorpions can be considered as a long-term solution for treatment against the *V. destructor*. However, their actual benefit is not entirely known and investigated thoroughly, and it is required to conduct field experiments. Another candidate for controlling *V. destructor* is another mite called *Stratiolaelaps scimitus*. However, research has shown that they sometimes prefer feeding on the honeybee eggs to the mite (96). Furthermore, there are also some field experiments revealing that they did not have enough effect to decrease the mites during early and late fall in the colonies. An efficient and desirable natural predator would have to eat the eggs of ectoparasites or the larvae directly to become a potential biological control candidate (20).

Microbiota: In the case of honeybees, worker guts from *V. destructor*-infested colonies have a high increased population of *Snodgrassella alvi* and a lower reduced population of *Lactobacillus spp.,* in comparison to un-infested colonies, showing that the acari has altered their microbiome (58, 74). Furthermore, un-infested larvae have an increased population of Enterobacteriaceae, whereas infected larvae have a diverse microbiota comparable to that of ectoparasites (26). As a result, the microbiota provides a new avenue for combating the ectoparasite. One of them believes that transgenic gut bacteria should be used for biocontrol. The symbiotic bacterium, *S. alvi*, from the honeybee gut can produce genes (12) that are transferred by conjugation to gut microbiota. Varroa feeding with designed bacteria expressed higher mortality in comparison to controls. It is, therefore, demonstrated that bees' gut bacteria can contribute to the better survival of parasitized honey bees.

Pathogens: *Bacillus thuringiensis*, an entomopathogenic microbe, is commonly employed as a bioinsecticide in agriculture (12, 66). It infects the host by ingesting a protein termed Cry, which creates crystals and vegetative poisons. *B. thuringiensis* was found on *V. destructor* corpses in *in vitro* investigations, and it was removed to assess its pathogenicity in mites and honeybees. It was found that *V. destructor* shook, regurgitated, suffered intestinal inflammation, and died after being treated with *B. thuringiensis* for 24 hours. Short-term exposure to *B. thuringiensis* had no lethal effects on *A. mellifera* adults or larvae, and it may have reduced vertical displacement, whereas chronic exposure to *B. thuringiensis* caused precocious mortality in both adults and larvae bees.

Entomopathogenic fungi, which destroy acarine species, were used in a number of experiments in addition to bacteria. Conidia are specialized spores that entomopathogenic fungi use to proliferate into their hosts. A lack of nutrients, water stress, toxic effect, and mechanical disruption require 3 to 10 days to kill the host. The fundamental disadvantage of fungus and bacteria, despite the lack of long-term data, is the low specificity of their toxins and the difficulty in colonizing and surviving in the hive ecology. Furthermore, from an evolutionary standpoint, the honeybee and the ectoparasite are related, making it difficult to isolate one from the other (3, 114).

Integrated Pest Management (IPM)

V. destructor is one of the most serious biological hazards to the health of western honey bees (*A. mellifera*

L.) globally. IPM is a method of keeping a pest or parasite population under control. Through the coordinated application of one or more procedures, an economic threshold can be reached. If the parasite density reaches a certain level, economic damage (loss of honey output or colony death) can be expected. Using chemical treatments and antibiotics need to be reduced in IPM programs, and they are avoided wherever possible. Minimizing chemical treatments ensures the integrity of hive products, increases the time it takes for parasites to develop resistance to treatments, and reduces the risk of detrimental effects on bees and the environment. The basic purpose of cultural control is to alter the hive environment to make it less conducive to the pest or illness while causing the honey bees as little harm as possible. Mechanical control includes pest control by physical methods or mechanical devices such as equipping the honeycomb with a sieve bottom plate, trapping broodstock with drones or heat treatment. These nonchemical approaches are considered essential for long-term, sustainable solutions to Varroa control (97).

The traditional definition of biocontrol is a pest management tactic that involves antagonistic organisms to reduce pest population densities (89). Varroa control is most commonly attempted using chemical treatment, though, within an IPM paradigm, the chemical application should be used in combination with other pest management methods to keep pest populations below economic injury levels or nuisance thresholds (34). Varroa mites continue to be a major issue for beekeeping despite efforts to control the problem. Sustainable control of Varroa is difficult to achieve using a single control approach, instead, it can be achieved by integrating multiple control approaches for maximum efficiency. However, because our understanding of how Varroa/virus transmission affects honey bees is low and our current economic threshold is narrow (2 vs > 3 mites/100 bees), it should be fair to consider IPM even as a viable approach to controlling Varroa (64).

Conclusions

V. destructor mite is the main pest that affects the health of developing and adult honey bees and, consequently, the entire honey bee colony. Honey bees have evolved individual and collective anti-parasite defense system including behavior systems in order to remove parasites from their body surface or from the parasitized brood. A variety of diagnostic and control methods including integrated Varroa control management practices are implemented in beekeeping operations. Diagnostic methods are used to establish the infestation levels in honey bee colonies in order to apply proper control methods, to minimize the use of chemically based acaricides. Sustainable control measures can be applied in organic and conventional beekeeping operations. The treatment methods include organic acids and essential oils. Applied IPM programmes combine a controlled and measured use of chemical treatments in order to ensure the integrity of hive products, lessen the risk of parasite resistance against acaricides developing and reduce the risk of detrimental effects on bees and the environment. To achieve sufficient efficacy of control treatments, applied biotechnical methods and the acaricidal substances need to be used in accordance with control protocol including, optimal time. Using recommended control methods, beekeepers avoid damage to adult bees and brood. In order to maximize mite control efficacy and to ensure production quality and safety of honey bee products, beekeepers need to consider seasonal treatment effects, medicinal product rotation and efficacy in Varroa control.

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Ethical Statement

This study does not present any ethical concerns.

Conflict of Interest

The authors declared that there is no conflict of interest.

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